REMARKS

Applicants have received and reviewed the Office Action dated May 23, 2001. Amendments to claims 25, 26, and 28 have been made to correct typographical errors. Claim 29 has been amended to more clearly define the subject matter and not for prior art purposes. Claim 33 has also been amended to correct the lack of an antecedent basis. No new matter has been added.

The outstanding Office Action has indicated that the rejection of claim 15 is withdrawn since Applicant's arguments have been found to be persuasive. Although the Office Action has concluded that no claim is allowed, there has not been any other rejection or objection applied to claim 15. Therefore, Applicant respectfully requests notice of allowability for the subject matter of claim 15.

35 U.S.C. §112, 1st paragraph

The Office Action has maintained the rejection of claims 23 and 38 under 35 U.S.C. §112, first paragraph as not being enabled by the specification. Specifically, the Office Action indicates that the specification does not enable the production of glycosylation site addition variants and that it would require undue experimentation by one of skill in the art to make and use the claimed invention. Applicants respectfully traverse this rejection.

Applicants submit that the specification provides both general and detailed support for adding a glycosylation site to a protein. Such general and detailed support can be found in the specification at least at page 2, lines 16-18; at page 3, lines 1-10; at page 7, lines 3-10 and 14-19; and at page 13, lines 1-16 and 20-25. In particular the specification lists many techniques, for example, oligonucleotide mediated (site-directed) mutagenesis, alanine scanning, PCR mutagenesis, cassette mutagenesis, and restriction selection mutagenesis (page 13, lines 20-25) which can be utilized for glycosylation site addition. Given this detailed of level description in the specification, one of skill in the art could insert sequences determining glycosylation sites into a protein to produce a glycosylation site addition variant. Furthermore, Applicant submits that these techniques were well known in the art at the time the application was filed and that undue experimentation would not be necessary for creating a glycosylation site addition variants.

Examples of methods for adding glycosylation sites to proteins can be found in many references published prior to the filing of the current application, for example, Melcher et al.

(1998), *Glycoconj J.* 10:987; Zhang and Salter (1998). *J. Immunol.* 160:831; and Leung et al. (1995), *J. Immunol.* 154:5919. For example, in Zhang and Slater, on page 832, in Materials and Methods, the paragraph on *site directed mutatgenesis and transfections* describes a process for adding glycosylation sites by using site-directed mutagenesis. In addition, as discussed in Leung et al. (*Int. J. Cancer* 60:534 (1995)), computer programs can be applied to perform molecular modeling of proteins to predict the accessibility of potential glycosylation sites.

Therefore, based on Applicants' disclosure and information known to those of skill in the art at the time of Applicants' disclosure, one of skill in the art can readily identify glycosylation addition sites and use techniques such as site directed mutagenesis to add a glycosylation site to the immunoadhesin.

These articles are provided for the Examiner's review and are intended to show that all of the methods needed to practice the invention are either described in the application or were known, and that there was a high level of skill in the art at the time the application was filed (see *In re Wands*, 858 F.2d 731, 8 USPQ2d 1400 (Fed. Cir. 1988)). It is also noted that the evidence provided by the applicant need not be conclusive by merely convincing to one skilled in the art (emphasis in original). MPEP 2165.05. Thus, the specification as filed fully supports claims reciting glycosylation site addition variants.

Applicant respectfully submits that, in light of the above, the rejection of claim 23 and 38 has been overcome and requests notification accordingly.

Claim 33 was rejected under 35 U.S.C. § 112, 2nd paragraph, as being vague and indefinite by use of inconsistent terminology. Applicants have amended this claim to correct the lack of an antecedent basis by using the term "segment". Applicant respectfully submits that, in light of the above, the rejection of claim 33 has been overcome and requests notification accordingly.

Claim 29 was rejected under 35 U.S.C. § 112, 2nd paragraph, as being vague and indefinite. Applicants have amended this claim to more clearly define the subject matter and not for prior art purposes. Applicant respectfully submits that, in light of the above, the rejection of claim 29 has been overcome and requests notification accordingly.

35 U.S.C. §103

The outstanding Office Action has maintained the rejection of claims 2-4 and 10-13 under 35 U.S.C. §103(a) as being unpatentable over Foster et al. (U.S. Patent No. 5,641,655; herein referred to as "Foster") and Ashkenazi et al. (*PNAS* 88:10535, 1988; herein referred to as "Ashkenazi"). Applicant respectfully traverses this rejection and indicates that the Examiner has not yet presented a *prima facie* case of obviousness. Applicant submits that claims 2-4 and 10-13 are not obvious over the combination of Foster and Ashkenazi, as discussed below, and the burden of proving the unpatentablility of these claims remains with the Examiner.

"To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations." M.P.E.P. §2143 at 2100-97. Applicant submits that the basic criteria are not met because there is no motivation in the references to modify the prior art.

As indicated in the Applicants' previous response of 28 February 2001, Foster does not discuss secretion of immunoadhesins or that a sequence including a pro-sequence of a mammalian t-PA could provide for secretion of an immunoadhesin or any other protein. Rather, Foster is directed to solving problems associated with thrombopoeitin (TPO) production. Specifically, TPO is subject to proteolysis and a need to produce TPO in large amounts in a cost effective manner was indicated as being desirable (column 2, lines 1-11). Foster indicated that TPO suffered from insufficient secretion and that by substituting the t-PA secretory peptide expression increased 5-10 fold (column 7, lines 33-44).

Ashkenazi does not discuss or suggest that secretion of the TNFR-IgG is problematic or needs to be increased, or that such an increase in secretion can be accomplished by using a sequence including a mammalian t-PA pro sequence. Neither Foster or Ashkenazi discusses secretion of immunoadhesins or the need to change the level of secretion of an immunoadhesin. Since Foster does not teach or suggest the use of a t-PA pro sequence to alter the secretion of a protein other than TPO, Ashkenazi would not have looked to Foster to modify the expression of TNFR-IgG.

Moreover, there is no teaching or discussion in either reference as to whether the t-PA signal/pro sequence would be properly processed and cleaved to provide for secretion of an immunoadhesin. The processing of a t-PA signal/pro peptide is complex (see pages 16-17 of the specification) and there is no teaching or suggestion that a t-PA signal/pro sequence would be properly processed when combined with an immunoadhesin. Therefore, one of skill in the art would have not have been motivated to combine the references cited by the examiner.

In the outstanding Office Action, the Examiner recognized that obviousness can only be established by combining or modifying the teaching of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so found either in the references themselves or in the knowledge generally available to one of ordinary skill in the art.

Additionally, the current invention provides unexpected results regarding the secretion of glycoproteins. As indicated on page 13, lines 14-21, of the substitute specification, deletion of N-linked glycosylation sites from a glycoprotein's secondary structure impaired the intracellular transport and secretion of the polypeptide. This impairment of secretion of these polypeptides was overcome by linking a mammalian (t-PA) pro peptide to the polypeptides. Surprisingly, and unexpectedly, it was found that secretion of the parent glycoprotein was also increased as well. The details of increased expression of TNFR-IgG1 can be found on page 29, lines 1-13, of the substitute specification.

Therefore, Applicants submit that this finding of unexpected results provides additional evidence that the Claims 2-4 and 10-13 are not obvious over Foster and Ashkenazi, taken alone or in combination.

In light of the above arguments and in absence of sufficient evidence to support a *prima facie* case of obviousness, Applicants respectfully request withdrawal of this rejection.

The outstanding Office Action has maintained the rejection of claims 2-5, 7-13 and further rejected claims 34-37 and 39-43 under 35 U.S.C. §103(a) as being unpatentable over Foster in view of Ashkenazi and Rickles et al. (*J. Biol. Chem.* (1988), 263:1563; herein referred to as "Rickles"). Applicant respectfully traverses this rejection. Applicant submits that claims 2-5, 7-13, 34-37 and 39-43 are not obvious over the combination of Foster, Ashkenazi, and Rickles, as discussed below, and the burden of proving the unpatentability of these claims remains with the Examiner.

The criteria for establishing a *prima facie* case of obviousness has been stated above. With regards to claims 2-5 and 7-13, Applicant submits that the basic criteria are not met because there is no motivation in the references to modify the prior art. With regards to claims 34-37 and 39-43, Applicant submits that the basic criteria are not met because in addition there being no motivation in the references to modify the prior art, the prior art references do not teach all the claimed limitations of the invention.

The Foster and Ashkenazi references are discussed above and the same arguments apply with respect to this rejection.

The Office Action has reiterated the argument that, "it would have been obvious for one of skill in the art to use the sequence for TNFR-IgG1 as the second DNA segment and the non-mammalian tPA prosequence disclosed by Rickles et al. in the constructs disclosed by Foster et al. to take advantage of the increased secretion rates associated with the t-PA pro chimeras disclosed by Foster et al."

As indicated in the Applicant's previous response of 28 February 2001, the deficiencies of the Foster and Ashkenazi references are not remedied by reference to Rickles. Rickles is directed to isolation and purification of a cDNA encoding a murine tissue plasminogen activator for use as a probe to analyze t-PA gene expression during F9 cell differentiation. There is no discussion or suggestion in Rickles that a sequence including a pro-sequence of a mammalian t-PA can or should be used to provide for secretion of any protein much less an immunoadhesin.

Furthermore, Claim 34 recites, "A DNA construct comprising a first DNA segment comprising a nucleic acid that encodes a mammalian t-PA prosequence operatively linked to a nucleic acid sequence that encodes a pre-sequence other than a mammalian t-PA pre sequence; and a second DNA segment operably linked to the first DNA segment, wherein the second DNA segment encodes a heterologous glycoprotein." As described in the specification at page 7, a prosequence of mammalian t-PA serves to target the polypeptide to the Golgi apparatus and a pre-sequence of mammalian t-PA directs the polypeptide to the lumen of the endoplamic reticulum.

Foster, Ashkenazi, and Rickles, have been discussed above and none of these references describe or suggest the use of a, "nucleic acid sequence that encodes a pre-sequence <u>other</u> than a mammalian t-PA pre-sequence." The Office Action has stated that, "Since Foster et al. disclose that **t-PAs from non-human sources** can be used in their method, and even listed an example

(see column 9 lines 5-9), it would have been obvious for one of skill in the art to use the sequence for TNFR-IgG1 as the second DNA segment and the non-mammalian prosequence disclosed by Rickles et al. in the constructs disclosed by Foster et al. to take advantage of the increased secretion rates associated with the t-PA pro chimeras disclosed by Foster et al." However, Rickles describes sequences from mouse t-PA (mammalian), but does not disclose a combining a mammalian t-PA prosequence with a pre-sequence other than a mammalian t-PA presequence. Foster, Ashkenazi, and Rickles are deficient in describing pre-sequence other than a mammalian t-PA pre-sequence and also are deficient in teaching or suggestion the combination of a pre-sequence other than a mammalian t-PA pre-sequence with a mammalian t-PA prosequence and a heterologous glycoprotein

With regards to claims 2-5, 7-13, 34-37 and 39-43, Foster, Ashkenazi, and Rickles, alone or in combination, provide no motivation to modify the prior art and therefore cannot be used to support a *prima facie* case of obviousness. With regards to Claims 34-37 and 39-43, Foster, Ashkenazi, and Rickles, alone or in combination, do not teach or suggest all the claimed limitations of the invention and therefore cannot be used to support a *prima facie* case of obviousness. Applicants respectfully request withdrawl of this rejection.

The outstanding Office Action has maintained the rejection of claims 2-4, 10-14, and 16-33 and has also rejected claims 34-46 under 35 U.S.C. §103(a) as being unpatentable over Foster in view of Ashkenazi et al. and Berman and Lasky (*Trends Biotech.* (1985), 3:1563; herein referred to as "Berman and Lasky").

The Foster et al., Ashkenazi et al., and Rickles references are discussed above and the same arguments apply with respect to this rejection.

The deficiencies of these references are not remedied by reference to the Berman and Lasky et al. reference. The Berman and Lasky et al. reference is a general reference that discusses the advantages of expression of fully glycosylated glycoproteins in mammalian cells, in particular, the expression of viral glycoproteins. There is no discussion in this very general reference of problems with secretion of glycoproteins nor that a sequence including a pro-sequence of a mammalian t-PA be operably linked to a DNA segment encoding an immunoadhesin or operably linked to a pre-sequence other than a mammalian t-PA pre-sequence. Furthermore, this reference does not teach or suggest the formation of glycosylation site variants. One of skill in the art reading the reference would not be motivated to prepare

glycosylation site variants because Berman and Lasky teach the advantage of fully glycosylated glycoproteins. Furthermore, Berman and Lasky is deficient in describing, "a nucleic acid sequence that encodes a pre-sequence other than a mammalian t-PA pre-sequence."

With regards to claims 2-4, 10-14, 16-33 and 34-46, Foster, Ashkenazi, Rickles, and Berman and Lasky, alone or in combination, provide no motivation to modify the prior art and therefore cannot be used to support a *prima facie* case of obviousness. With regards to Claims 34-46, Foster, Ashkenazi, Rickles, and Berman and Lasky, alone or in combination, do not teach or suggest all the claimed limitations of the invention and therefore cannot be used to support a *prima facie* case of obviousness. Applicants respectfully request withdrawl of this rejection.

Thus, Applicants respectfully request withdrawal of the rejection.

In light of the foregoing amendments and remarks, it is believed that the application is in condition for allowance. A notification to that effect is earnestly solicited. The Examiner is invited to telephone Applicants' undersigned representative at the telephone number listed below if the Examiner believes that doing so will advance prosecution of this application.

Respectfully submitted,

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MARKED-UP VERSION SHOWING CHANGES

25. The DNA construct of claim 24 wherein the heterologous glycosylation site variant is an [immunoadhesion] immunoadhesin.

26. The DNA construct of claim 25 wherein the [immunoadhesion] immunoadhesin is TNFR-IgG.

28. The DNA construct of claim 27 wherein the TNFRI-IgG has an N-linked [glycostation] glycosylation site selected from the group consisting of amino acid positions 14, 105, 111 and 248 deleted.

29. The DNA construct of claim 28 wherein the TNFRI-IgG has the N-linked site at amino acid position 14 deleted.

33. A method of producing a polypeptide which has been altered to delete one or more native N-linked glycosylation sites comprising the steps of

(a) culturing a eukaryotic host cell comprising a DNA construct comprising:

first DNA segment encoding a precursor peptide corresponding to a mammalian tissue plasminogen activator signal-pro peptide; and

a second DNA segment operably linked to the first DNA [sequence] segment, the second DNA [sequence] segment encoding a heterologous glycosylation site deletion variant polypeptide; wherein the eukaryotic host cell express the first and second DNA segments and the polypeptide is secreted from the cell; and

(b) recovering the polypeptide so produced.